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Syntheses and antitumor activity of some 1,2,4 – triazine derivatives

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Abstract:-The 2,3,5- tri substituted –1,2,4triazin -6 - ones (**2**,**3**) are synthesized via condensation of 2- phenyl - 4 - (4-Floro benzylidene) 1,3-oxazol -5- one (**1**) with hydrazine hydrate, semicarbazide, and thiosemicarbazide, followed by cyclization with removal of water molecule. Acetylating compounds **2**,**3** with acetic anhydride yields the diacetyl derivative **4** and fused triazolo - 1,2,4triazine derivative **5**, respectively. Treatment of compound **3**a with ethyl chloro acetate in the presence of fused sodium acetate gives the corresponding fused 1,2,4-triazine derivative **6**. 3- acetyl- 5,5-di substituted -1,2-dihydro - 1,2,4- triazino-1,2,4-triazin - 1,4,8-triones **7** was prepared via acetylating compound **6** with acetic anhydride under reflux. The structure of the compounds were characterized based on their spectral data. The cyotoxic activities of the prepared compounds have been studied on the tumor cell line human colon carcinoma (HCT-116) cell using the MTT viability test.

Keywords: 1,2,4-triazine derivatives, Hydrazine hydrate, semicarbazide, thiosemicarbazide, anti tumor activity against HCT-116 cell line.

I. INTRODUCTION

1,2,4-triazine derivatives have been widely studied in terms of their synthetic methodologies and reactivity since some of these derivatives were reported to have promising biological activities such as kinase inhibition activities, anti microbial activity , inflammatory, analgesic, antiviral, anthelminitic activities [1-7]. The synthesis of 1,2,4-triazine derivatives have been documented and their methods of preparation were modified and varied. A survey of the literature [8-10] reveled that oxazolinone compounds are the most common reagents used for the synthesis of 1,2,4-triazine derivatives. The objectives of the present work were the syntheses , spectroscopic characterization of 1,2,4-triazine derivatives by using the oxazolinone derivatives and hydrazines then studying the cyotoxic activities of the 1,2,4,-triazine derivatives against human colon carcinoma cell line using the MTT viability test .

II. RESULTS AND DISCUSSION

CHEMISTRY

Oxazolinone and related compounds have been described as useful building blocks for the assembly of various heterocyclic rings.

We have reported the synthesis of 2- phenyl - 4 - (4-Floro benzylidene) 1,3- oxazol -5- one (**1**) via condensation of 4-Floro benzaldehyde with N-benzoyl glycine in presence of fused sodium acetate and acetic anhydride under fusion according to the literature method [11]. Treatment of 2- phenyl - 4 - (4-Floro benzylidene) 1,3-oxazol -5- one **1** with nitrogen nucleophilic reagents such as (hydrazine hydrate, semicarbazide hydrochloride, and thiosemicarbazide) in glacial acetic acid under reflux lead to the formation of 2-substituted -3- phenyl -5- (4-Floro benzylidene) -1,2,4-triazin-6- ones (**2** and **3**;Scheme 1)[12,13] .

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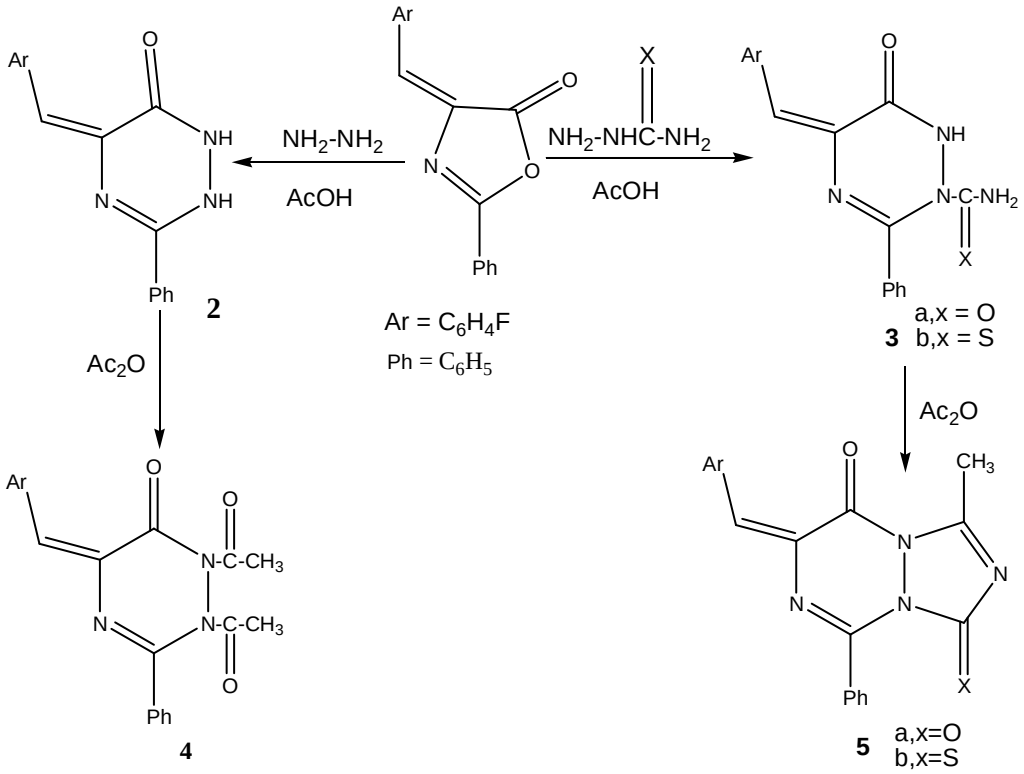

Scheme 1: Syntheses of compounds **2,3** (a,b)**, 4, 5** (a,b)

Acetylating 3- phenyl -5- (4-Floro benzylidene) -1,2 -dihydro-1,2,4-triazin -6- one **2**, 2- (amino) carbonyl -3-phenyl-5-(4-Florobenzylidene)- 1,2,4-triazin -6- one **3**a and 2-(amino) thiocarbonyl -3-phenyl-5-(4-Florobenzylidene)- 1,2,4-triazin -6- one **3**b with acetic anhydride under reflux lead to the formation of the corresponding 1,2-di acetyl -3- phenyl -5-(4-Floro benzylidene) -1,2,4-triazin -6- one **4** , 1-methyl-3-oxo-4-phenyl -6-(4-Floro benzylidene) – triazolo [2,1a] -1,2,4-triazin -7- one **5**a and 1-methyl-3-thioxo-4-phenyl -6-(4-Floro benzylidene)-triazolo [2,1a] -1,2,4-triazin -7- one (**5**b;Scheme1).

Recently we have reported the syntheses of fused triazino - 1,2,4 triazine-8-one by condensation of 2-amino thiocarbonyl-1,2,4-triazin-6-one with ethyl chloro acetate in presence of fused sodium acetate, followed by cyclization with removal of ethanol molecule[14]. Based on the same principle ,we described her the synthesis of 5-phenyl -7- (4-Floro benzylidene) -1,2-dihydro-1,2,4,-triazino-[2,1-a]-1,2,4-triazin- 1,4,8-triones **6** starting from 2-aminocarbonyl -3-phenyl-5-(4-Florobenzylidene)- 1,2,4-triazin -6- one **3**a and ethyl chloro acetate in the presence of fused sodium acetate in glacial acetic acid under reflux.

Acetylating 5-phenyl -7- (4-Floro benzylidene)-1,2-dihydro-1,2,4,-triazino-[2,1-a]-1,2,4-triazin-1,4,8-triones **6** with acetic anhydride gave the corresponding 3-acetyl -5- phenyl-7-(4-Floro benzylidene)-1,2-dihydro-1,2,4,-triazino-[2,1-a]-1,2,4-triazin-1,4,8-triones (**7** ;Scheme: 2).

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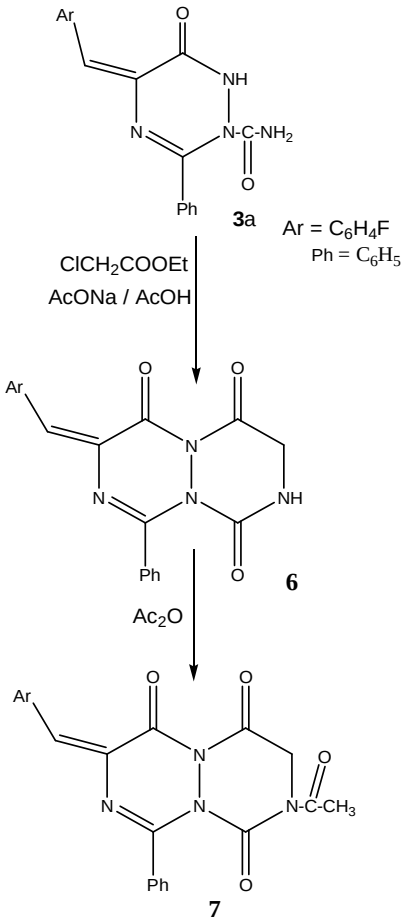

Scheme 2: syntheses of compounds **6,7**

ANTITUMOR ASSAY.

The cyotoxic and antitumor activities of the synthesized compounds **3-7** were tested against the HCT-116 cell line according to method of Mossman [15], Gandadevi and Muthumary [16]. The inhibitory activities against Human colon carcinoma (HCT-116) cell was detected by using different concentrations of the tested compounds (50, 25, 12,5, 6.25, 3.125 and 1.56 mg) and the viability cells (%) was determined by the colorimetric method. The drug doxorubicin was used as standard.

The 50% inhibition concentration (IC_{50}) of the (HCT-116) cell line was calculated from (Table **1** and Figure **1,2**)

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Sample conc. (μg)	Viability%						
	3a	3b	4	5a	6	7	Doxorubicin
50	48.16	4.72	7.93	7.81	24.59	5.05	6.82
25	83.42	9.08	13.27	13.24	76.38	9.74	8.89
12.50	94.15	13.74	22.34	21.36	87.65	14.87	14.83
6.25	98.96	22.37	68.36	29.58	95.82	22.04	16.16
3.125	100	37.82	89.65	52.19	98.74	40.56	22.28
1.56	100	54.21	96.12	79.03	100	74.98	34.64
0	100	100	100	100	100	100	100
IC_{50}	48.70	1.96	8.74	3.43	37.70	2.69	0.469

Table **1**:Evaluation of cyotoxicity of the synthesized compounds against (HCT-116) cell line.

IC50 (□g) values of the synthesized compound after 72 hr. continuous exposure of tumor cell line.

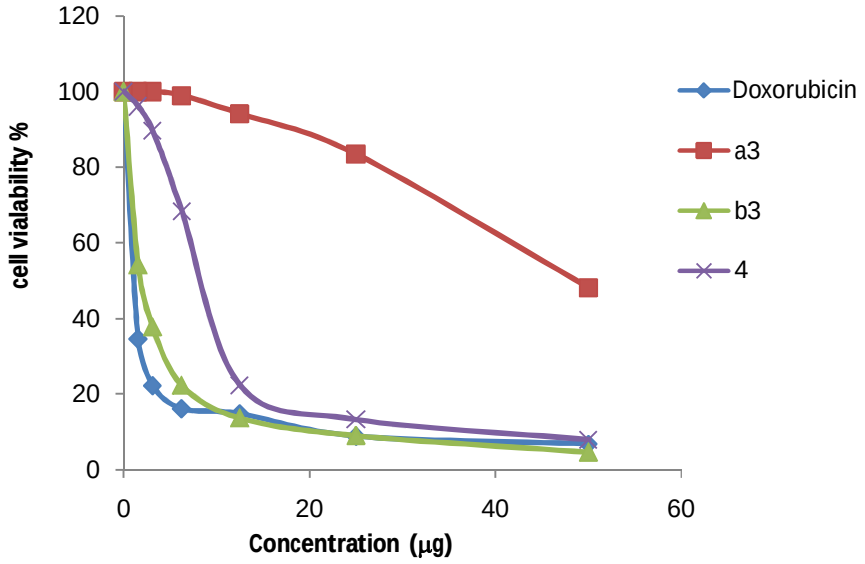

Figure 1: Evaluation of cyotoxicity of compounds (3a, 3b,4).

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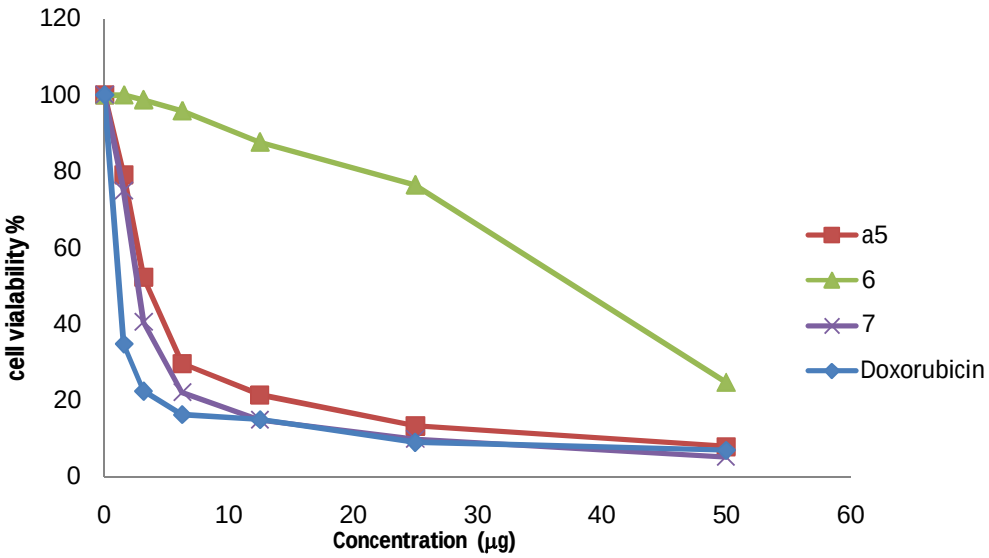

Figure 2: Evaluation of cyo toxicity of compounds (5a, 6,7).

The IC_{50} value is the concentration that induces 50% growth inhibition compared with untreated control cells.
Compounds **3**b , **5**a and **7** were found to have activity near from the standard antitumor drug Doxorubicin against Human colon carcinoma (HCT-166) cell line.
In comparison with standard antitumor drug Doxorubicin, compounds **3**a and **6** were observed to be more weakly active against (HCT-116) cell line, while compound **4** was found to has moderate activity against (HCT-116) cell line.

III. EXPERMENTAL RESULTS

Melting points were taken in open capillaries with a Thomas uni-melt apparatus and they are uncorrected. ^{1}HNMR spectra were recorded on a ^{13}CNMR (500 MHz) spectra were run in di methyl sulfoxide(DMSO-d6). Chemical shifts were related to that of the solvent. IR spectra were recorded on a Perkin-Elmer 1420 spectrometer and a Bio-Rad FTS7 (KBr). Mass spectra were obtained on a Joel JMS D-300 spectrometer operating at 70 Ev. Microanalyses were conducted using an elemental analyzer 1106 and it is within ±0.5of the theoretical values . general electric QE300 instrument and chemical shifts are given with respect to TMS.

2-Substituted-3-phenyl-5-(4-florobenzylidene)-1,2,4-triazin-6-ones (2 and 3 a, b**)**
A mixture of oxazolinone (**1**,0.01 mol) and hydrazine hydrate derivatives (namely ,hydrazine hydrate , semicarbazide and thiosemicarbazide) (0.01 mol) in (30 ml) glacial acetic acid were heated under reflux for 2-3 hr. the reaction mixture was cooled and poured on cold water ,the solid obtained was filtered off, washed with water ,dried and purified from ethanol to give(**2, 3** a, b).
3-Phenyl-5-(4-florobenzylidene)-1,2-dihydro-1,2,4-triazin-6-ones (2), as pale yellow crystals, yield 71%, m.p. 210 ^{O}c. IR (KBr): 3221(NH), 1695(C=O), 1632(C=N), 1605,1595(C=C) cm^{-1}. ^{1}H NMR (DMSO-d6)δ: 7.36 - 8.411 (m, 10H, Ar-H and H- olefinic), 10.21 (s, 1H, NH), 10.31(s, 1H, NH) ppm. ^{13}C- NMR (DMSO-d6) δ: 163.61 (C=O), 162 (C=N), 135.34, 135.25, 134.21, 133.21, 130.61, 130.58, 129.89, 129.83, 129.22, 128.47, 125.55, 116.79, 115.60 (C-Ar) ppm.MS: m/z (%) = 281 (44.74), 282 (M $^{+}$+1, 9.14), 77 (100). Anal. Calcd. For $C_{16}H_{12}N_3FO$: C,68.33: H,4.27; N,14.34. Found C,68.03; H,4.13; N,14.21.

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2-(Amino) carbonyl -3-phenyl -5-(4-florobenzylidene)- 1,2,4-triazin -6- one (3a), as yellow crystals, yield 73%, m.p.215 ^{O}c. IR (KBr):3325, 3187 (NH_2), 3210(NH), 1705-1698 (br.CO), 1631(C=N), `1607,1589(C=C)cm^{-1}. . ^{1}H NMR (DMSO-d6)δ: 7.31-8.21 (m, 10 H, Ar-H and H- olefinic), 8.42 (s,2H,NH_2), 10.32 (s, 1H,NH) ppm. ^{13}C- NMR (DMSO-d6) δ: 169.23 (C=O), 164.71(C=O), 162.33 (C=N), 136.61, 134.23, 132.50, 131.22, 129.83, 129.15, 129.04, 128.84, 128.48, 128.13,126.77, 125.56, 116.80, 116.59 (C- Aromatic) ppm. MS: m/z(%) = 324 (75.58), 326 (M^+ +2, 31.2),325 (M^+ +1,72.09), 310(91.86). Anal. Calcd. For $C_{17}H_{13}N_4FO_2$: C,62.96; H,4.01; N, 17.28. Found: C,62.66; H,3.97; N,12.09. .

2-(Amino) thiocarbonyl -3-phenyl-5-(4-florobenzylidene)- 1,2,4-triazin -6- one (3b) as yellow crystals, yield 75%, m.p. 215 ^{O}c. IR(KBr): 3321, 3159 (NH_2), 3210 (NH), 1695 (C=O), 1632(C=N), 1608, 1593(C=C), 1358(C=S) cm^{-1}. . ^{1}H NMR (DMSO-d6)δ: 7.32-8.11 (m, 10 H, Ar-H and H-olefinic), 8.33(s,2H, NH_2), 10.06(s,1H,NH) ppm. ^{13}C- NMR (DMSO-d6) δ:176.32(C=S), 167.28(C=O), 162.13(C=N), 136.61, 135.35, 135.26, 134.22, 132.50, 131.25, 129.99, 129.15, 129.04, 128.84, 128.13, 126.77, 116.59, 116.38, (C-Aromatic) ppm. MS: m/z (%) = 340 (13.81), 342 (M^+ +2, 5.131), (M^+ +1, 7.86), (M^+ -1, 7.13.80), 77(100). Anal.Calcd. For $C_{17}H_{13}N_4FSO$: C,60.00; H,3.82; N,16.47; Found: C,59.93; H,3.63; N,16.24.

Syntheses of 1,2-di acetyl -3- phenyl -5-(4-floro benzylidene) -1,2,4-triazin -6- one ***(4)*** *, 1-methyl-3-oxo-4-phenyl -6-(4-Floro benzylidene)- triazolo [2,1a] -1,2,4- triazin -7- one* ***(5a)*** *and 1-methyl-3-thioxo-4-phenyl-6-(4-florobenzylidene) triazolo[2,1a]-1,2,4-triazine-7-one (****5b****)*

A solution of (**2**) and/or (**3**) (0.01mole) in acetic anhydride (25ml) was heated under reflux for 2 hrs and poured into ice-water. The resulting product was filtered off, washed with water, dried and purified by recrystalization from ethanol to give (**4** and **5**a,b).

1,2 - Di acetyl -3- phenyl -5- (4-Floro benzylidene) - 1,2,4-triazin- 6 - one (4), as pale yellow crystals, yield 67%, m.p.170 ^{O}c. IR(KBr) 1705-1689 (br.C=O), 1631(C=N), 1607,1593 (C=C) cm^{-1}. ^{1}H NMR (DMSO-d6)δ: 2.49-2.50 (s, 6H, 2CH_3), 7.35-8.41 (m, 10H, Ar-H and H-olefinic) ppm. ^{13}C- NMR (DMSO-d6) δ: 167.33, 165.26, 163.16 (C=O), 162.31 (C=N), 135.08, 135.20, 134.96, 134.22, 133.22, 130.71, 130.59, 129.98, 129.51, 128.48, 128.11, 127.03, 125.56, 116.80 (C –aromatic), 24.99 (CH_3),24.99 (CH_3) ppm. MS: m/z (%) 365(24.25) ,367 (M^++2,12.20), 366 (M^+ +1,.20.15), 123(89.93). Anal. Calcd. For $C_{20}H_{16}N_3FO_3$: C,65.75; H,4.83; N,11.51; Found: C,65.55; H,4.17; N,11.33.

1-Methyl-3-oxo-4-phenyl-6-(4-florobenzylidene) – triazolo [2,1a] - 1,2,4-triazin -7- one (5a), as yellow crystals, yield 63%, m.p. 160 ^{O}c**.** IR (KBr): 1705-1697 (br. C=O), 1631 (C=N), 1608,1543 (C=C)cm^{-1}. ^{1}H NMR (DMSO-d6)δ: 2.51 (s, 3H, CH_3), 7.30-8.42 (m,10H,Ar-H and H-olefinic) ppm. ^{13}C- NMR (DMSO-d6) δ: 169.31, 163.30 (C=O), 162.22, 161.30 (C=N), 136.50, 134.32, 132.52, 131.23, 129.81, 129.14, 129.03, 128.86, 128.44, 128.12, 126.75, 125.53, 116.80,116.53 (C-aromatic), 27.21 (CH_3) ppm. MS: m/z (%): 348 (70.73), 350 (M^+ +2,21.10),349 (M^+ +1, 67.07),336 (95.12). Anal. Calcd. For $C_{19}H_{13}N_4FO_2$: C,65,52 ; H,3.73; N,16.09; Found: C,65.25;H,3.48; N,16.01.

1- Methyl -3- thioxo-4-phenyl -6- (4-Floro benzylidene) - triazolo [2,1a] - 1,2,4- triazin-7- one (5b), as yellow crystals, yield 66%, m.p. 167 ^{O}c**.** IR (KBr): 1695 (C=O), 1633 (C=N), 1610,1593 (C=C), 1356 (C=S) cm^{-1}. ^{1}H NMR (DMSO-d6)δ: 2.53 (s, 3H, CH_3), 7.29-8.31 (m,10H,Ar-H and H-olefinic) ppm. ^{13}C- NMR (DMSO-d6) δ: 172.30 (C=S),163.3 (C=O), 162.11, 161.83 (C=N), 136.23, 134.21, 132.53, 131.22, 129.83, 129.20, 129.05, 128.83, 128.41, 128.13, 126.73, 125.55, 116.82, 116.53 (C-aromatic and C- olefinic.), 27.24 (CH_3) ppm. . MS: m/z (%): 364 (33.20), 366 (M^+ +2, 12.20),365 (M^+ +1, 11.30), 186 (100). Anal. Calcd. For $C_{19}H_{13}N_4FOS$: C,62,74 ; H,3.75; N,15.38; Found: C,62. 57; H, 3.43; N,15.22.

Synthesis of 5-phenyl -7- (4-floro benzylidene) -1,2-dihydro-1,2,4,-triazino-[2,1-a]-1,2,4-triazin- 1,4,8-triones ***(6).***

A mixture of **3**a (0.01mole) and ethyl chloro acetate (0.01 mol) in acetic acid (30 ml) in presence of fused sodium acetate (0.03 mol) was heated under reflux for 3hrs.then cooled and poured into water. The resulting solid was filtered off, washed with water, dried and purified by crystallization from acetic acid to give **6** as yellow crystals, yield 73%, m.p.235 ^{O}c**.** IR (KBr): 3227 (NH), 1706-1693 (br.CO), 1633 (C=N), 1612,1605 (C=C) cm^{-1}. ^{1}H NMR (DMSO-d6)δ:3.53 (s, 2H, NCH_2CO), 7.25-8.41 (m,10H,Ar-H and H-olefinic), 10.35 (s, 1H, NH) ppm. ^{13}C- NMR (DMSO-d6) δ: 168.33, 167.22, 163.41 (C=O), 162.16 (C=N), 135.80, 135.25, 134.93, 134.21, 133.22, 130.26, 130.50, 129.98, 129.53, 128.47, 128.18, 127.03, 116.79, 116.54 (C-aromatic and C- olefinic.), 40.61 (CH_2) ppm. . MS: m/z (%): 364

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(45.73), 366 (M^+ +2, 37.16),365 (M^+ +1, 39.19), 105 (100). Anal. Calcd. For $C_{19}H_{13}N_4FO_3$: C,62,64 ; H,3.57; N,15.36; Found: C,62. 52; H, 3.49; N,15.19.

Synthesis of 3-acetyl -5- phenyl-7-(4-floro benzylidene)-1,2-dihydro-1,2,4,-triazino-[2,1-a]-1,2,4-triazin-1,4,8-triones (7).

A solution of **6** (0.01mol) in acetic anhydride (20 ml) was heated under reflux for 2hrs. then cooled and poured onto ice-water. The solid obtained was filtered off, washed with water, dried and purified by crystallization with ethanol to give **7** as pale yellow crystals, yield 68%, m.p. 235 ^{O}c**.** IR (KBr): 3227 (NH), 1710-1695 (br.CO), 1635 (C=N), 1610,1603, 1589 (C=C) cm^{-1}. ^{1}H NMR (DMSO-d6)δ:2.41(s, 3H, CH_3), 3.43 (s, 2H, NCH_2CO), 7.31-8.35 (m,10H,Ar-H and H-olefinic) ppm. ^{13}C- NMR (DMSO-d6) δ: 170.84, 168.43, 167.33, 165.31(CO),162.81 (C=N), 135.89, 135.34, 134.22, 133.22, 10.62, 130.59,129.98, 129.51, 128.48, 128.18, 127.03, 125.56, 116.80, 116.58 (C-aromatic and C-olefinic.), 41.62 (CH_2) ppm. . MS: m/z (%): 406 (8.36), 408 (M^+ +2, 26.31),407 (M^+ +1, 8.65), 119 (100). Anal. Calcd. For $C_{21}H_{15}N_4FO_4$: C,62,07 ; H,3.57; N,13.97; Found: C,61. 97; H, 3.36; N,13.53.

IV. CONCLUSION

The researches study the successful synthesis and antitumor activity of some1,2,4-triazine derivatives. The investigation of antitumor activity revealed that compounds **3**b**, 5**a and **7** are most potent against Human colon carcinoma (HCT-166) cell line, IC_{50} (1.96), IC_{50} (3.43), IC_{50} (2.69) respectively . While compound **4** has moderate activity , compounds **3**a and **6** have weak activity. Hence oxo and thioxo derivatives have good antitumor activity**.**

REFERENCES

[1] Kidwai,M.; Goel,Y.; Kumar,R.; Indian J.Chem.,37B, 174 (1998).
[2] Holla,B.S.; Gonsalves,R.; Rao,B.S.; Gopala Krishna,H.N.; Farmaco,56,899 (2001).
[3] Abdel Rahman,R.M.; Morsy,J.M.; Hanafy,F.;Amene,H.A.; Pharmazie,54,347 (1999).
[4] Partidge,M.W.; Stevens,M.F.G.; Chem.Soc., 1127 (1966).
[5] Abd,E.I.; Samii,Z.K.; J.Technol.Biotechnol., 53,143 (1992).
[6] Hay,M.P.; Prujin,F.B.; Gumage,S.A.; Liyomage,H.D.; Wilson,W.R.; J.Med.Chem., 47,475 (2009).
[7] Sztanke,k.;Fidecka,S.; Kedzierska,E.; Karczmarzyk,Z.; Pihlaja,K.; Matosiuk,D.; Eur.J.Med.Chem.,40,127 (2005).
[8] Boger,D.L.; Chemical Reviews, 86, 781 (1986).
[9] Khramou, D.M.; Bielawski,G.W., Chemical Communications,49,58 (2005).
[10] Benson,S.G.; Synder,J.K.; J.Org.Chem.,85,57 (1992).
[11] El-Deen,I.M.; Chinese J.Chem.16 (6), 533 (1998).
[12] El-Sakka,s.s.; Soliman,A.H; Imam,A.M.; Afinidad,3,539 (2009).
[13] Dawane, B.S.; Kadam,N.S.; Shaikh,MB.; Der Pharma Chemica let.,2(4),126 (2010).
[14] Hassenen,J.A.; Abd-El Moneum,M.; El-Deen, I.M.; Res Chem.intermed., (A vilable on line).
[15] Mosmann,T.; J.Immunol. Methods,65,55 (1983).
[16] Gangadevi,V.; Muthumary, J.; African J. of Biotechnology, 6, 1382 (2007).